# Some Observations on the Microscopy of Lard and Rearranged Lard[1]

S. F. HERB, M. C. AUDSLEY, and R. W. RIEMENSCHNEIDER, Eastern Regional Research Laboratory,[2] Philadelphia, Pennsylvania

THE CRYSTALLINE NATURE of glyceride mixtures, such as occur in natural fats, is an important though complex problem for study. The physical properties of a fat are closely related to the size and shape of the crystals and the tendency of those crystals to vary with methods of production, tempering, and conditions of storage of the fat.

A review of the literature shows that considerable but not extensive use has been made of microscopy in connection with various phases of fat chemistry and technology. Work prior to 1936 is adequately presented by Mehlenbacher (24) and Greene (8). In general, these early investigations were conducted on fats and oils crystallized from solvents or on derivatives of fatty acids in efforts to identify a particular fatty component. For example, Reinders, Doppler, and Oberg (28) concluded, from microscopic examination, that cocoa butter was either a single pure glyceride or a mixture of two or more glycerides which do not crystallize separately but in a solid state form homogeneous mixed crystals. Dilatometric studies and fractional crystallization of the fat confirmed the latter conclusion. The literature since 1936 shows further useful applications of microscopy in the chemistry of fats. Green (9—16) has published photomicrographs of the reaction products of fats with alkalies and phenylhydrazine. Weitkamp (31) has recorded dark-field photomicrographs of n-fatty acids, optically active 2-hydroxy acids, iso acids, and dextrorotary anteiso acids obtained from degras. Rosevear (30) has presented an excellent paper on the microscopy (polarized light) of liquid crystalline neat and middle phases of soaps and synthetic detergents. Ravich and coworkers (1, 7, 26, 27) employed polarized light microscopy in studying polymorphism, solid solutions, and thermal behavior of various triglycerides and fatty acids. Malkin *et al.* (4, 5, 6, 21, 22, 23) and Quimby (25) have employed polarized light illumination to observe various known glycerides and their polymorphic forms.

While microscopic examination has been employed rather widely in the field of fats and oils, as previously noted, very little has been published on the crystal habit of shortenings. Bailey (2) presented several photomicrographs of various shortenings. Hellman, Zobel, and Senti (17) reported certain changes in global spread caused by tempering. Hoerr and Waugh (20) found that certain differences between lard and rearranged lard are revealed by means of microscopic examination that are not shown by the usual analytical methods.

Recent developments and improvements in microscopes and methods of illumination have extended the adaptability and usefulness of the instrument to the study of many problems. Phase contrast illumination is one of these recent developments which is finding wide usage in the microscopical study of transparent or relatively transparent specimens in which detail is not rendered satisfactorily by conventional methods (3). Reynolds and Verma (29), using phase contrast and polarized light illumination, made observations on the growth and optical properties of stearic acid crystals grown from a dilute solution of benzene. Hessler (18, 19) has compared phase contrast with polarized light illumination in an investigation of the structure of waxes and their identification and concluded that phase contrast microscopy would be useful in further work. In the present investigation, phase contrast and polarized light microscopy were employed to observe the crystal formations of lard and rearranged lard under different conditions of sample preparation and treatment.

## Experimental

*Equipment.*[3] In this investigation an American Optical Company petrographic microscope was employed for obtaining the photomicrographs with polarized light. Illumination was furnished by a "Zirconarc" photomicrograhic lamp made by the Fish-Shurman Corporation.

A Bausch and Lomb microscope equipped with B. & L. turret-type Abbe condenser phase contrast accessories and illumination by means of a B. & L. Research Illuminator equipped with a ribbon filament bulb and a green filter (Wratten "B" type, No. 58) were employed for photomicrographs in phase contrast.

All photomicrographs were taken with a B. & L. camera, Type H, using 4 x 5 inch Ortho-X cut film.

*Techniques.* The preparation of slides for microscopic examination is of utmost importance, and numerous techniques were tried in order to obtain satisfactory reproducibility of slides and crystal appearance.

Three general techniques or procedures proved best for their particular purpose and were used in this work:

a) A definite amount of a plasticized sample was weighed on a slide, an 18-mm. diameter circular cover glass was placed

[1] Presented at the meeting of the American Oil Chemists' Society, Philadelphia Pa., Oct. 10–12, 1955.
[2] A laboratory of the Eastern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture.
[3] Mention of specific firms and products throughout this paper does not imply endorsement of such by the Department of Agriculture to the possible detriment of similar firms and products not mentioned.

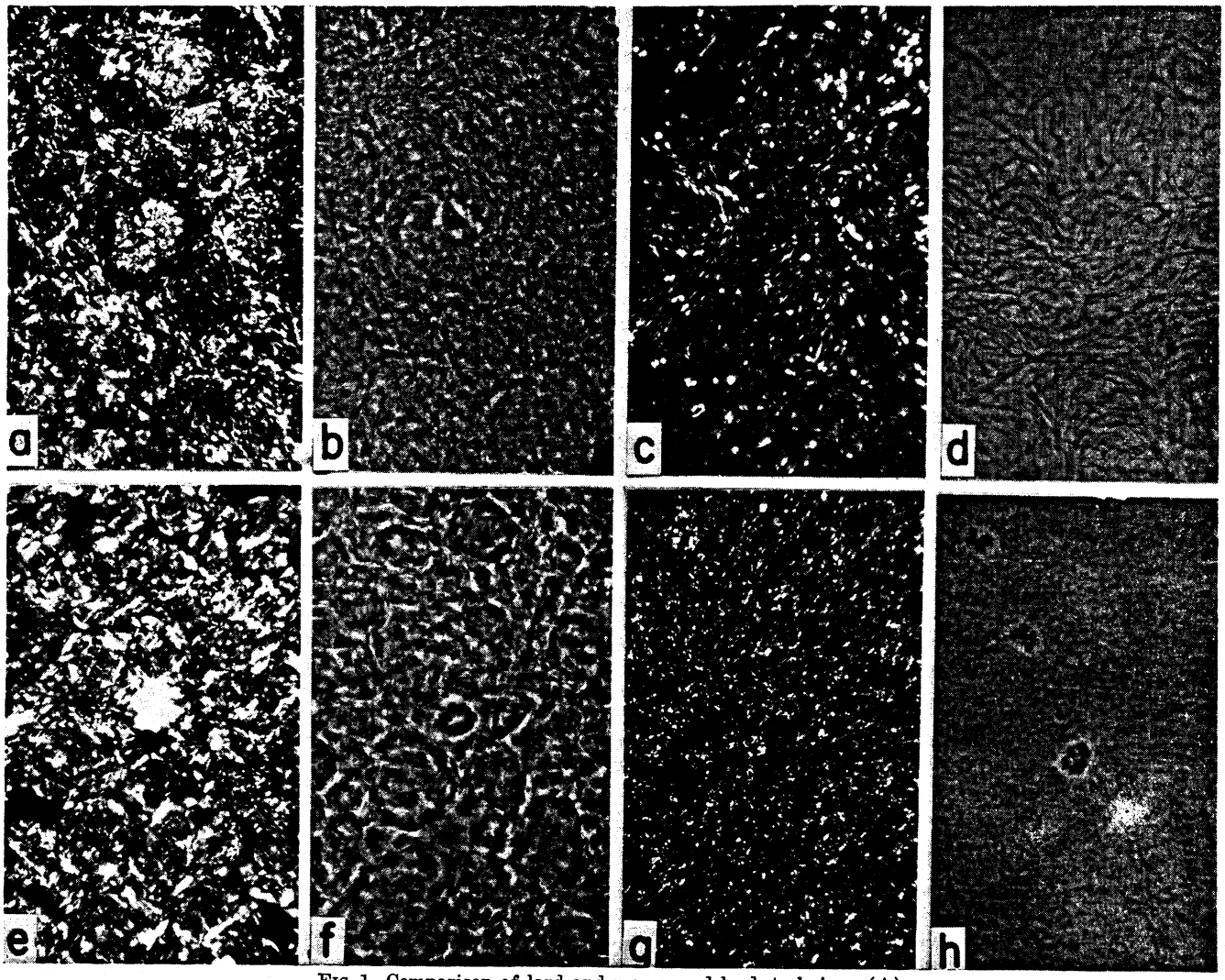


FIG. 1. Comparison of lard and rearranged lard, technique (A).

Plasticized lard, 400 x magnification
(a) Thick preparation, polarized light
(b) Thick preparation, phase contrast
(c) Thin preparation, polarized light
(d) Thin preparation, phase contrast

Plasticized rearranged lard, 400 x magnification
(e) Thick preparation, polarized light
(f) Thick preparation, phase contrast
(g) Thin preparation, polarized light
(h) Thin preparation, phase contrast

on the sample, and pressure was applied until the sample was spread just to the edge of the cover glass in all directions. The weight of sample varied from about 2 to 25 mg., depending on whether a thin or thick preparation was desired. The prepared slides were stored and examined periodically at a temperature of 24.5°C.

b) This procedure was similar to that employed by Hoerr and Waugh (20) except that thinner slide preparations were used. About 2.5 mg. of plasticized fat were pressed out to the edge of the cover glass as in a), the slide was warmed (about 45°C.) to melt the sample and then held in a constant temperature room at 24.5°C. to allow fat crystals to develop. Examination of the slide prepared in this manner will almost always show areas toward the edge of the cover glass which contain fewer crystals, and it is these areas which are best suited for study. The pressing of the plasticized sample appears preferentially to move the oil phase toward the edge of the cover glass, thereby effecting somewhat of a dilution of the crystals in this region. Upon examination of a specific field in this region over a period of days, changes can be observed and recorded by photomicrographs. All microscopic examinations and photomicrographs were made in the constant-temperature room.

c) Larger crystals and better definition of crystal shape can be obtained by diluting the fat sample. The choice of diluent is limited because it must dissolve the oil phase but not affect the crystals. A diluent composed of oil from a portion of the sample to be examined was prepared by filtering some of the oil phase from the plasticized sample which had been held at least 48 hrs. at 23°C. The diluent obtained in this way should not crystallize when held at 24.5°C., the temperature used in the microscopical examinations. The dilution of the sample and preparation of the slide was accomplished in the following manner. One part of sample and 8 parts of diluent oil were weighed in a small beaker and, after warming to melt sample, were mixed thoroughly. About 3.5 mg. of this mixture were weighed on a slide and covered with a cover glass (18 mm. in diameter). Slight pressure may be necessary to spread the melted mixture uniformly to the edge of the cover glass. The prepared slide was then stored at 24.5°C. for crystals to develop and for microscopical examination.

## Results and Discussion

*Observations with Technique (A)*: Differences in crystal appearance were observed between thick and thin preparations of the same sample and also between lard and rearranged lard as shown in Figure 1. In making the thin preparations, the crystals or crystal aggregates may be broken up, and this may account for the differences in appearance between these and the thick preparations. Long thread-like needles are apparent in the photomicrographs of lard with phase contrast (b, d), whereas in polarized light the crystals appear as clumps (a) or as short needles (c). When similar preparations of lard and rearranged lard are compared, *i.e.*, thick with thick and thin with thin, the crystals of rearranged lard (e, f, g, h) appear

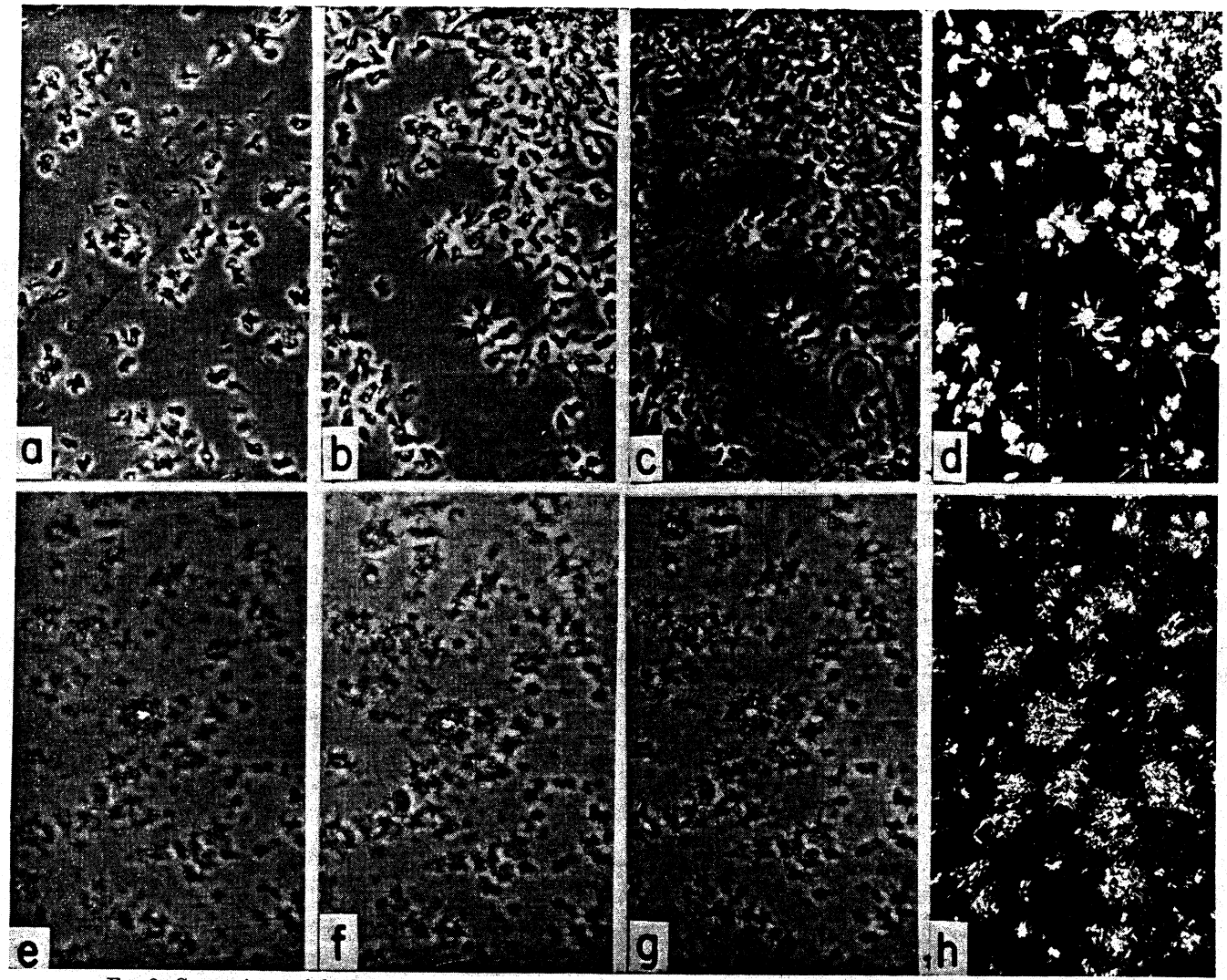


FIG. 2. Comparison of lard and rearranged lard after different storage periods at 24.5°C., technique (B).

| Lard, 400 x magnification | Rearranged lard, 400 x magnification |
|---|---|
| (a) 2 hours, phase contrast | (e) 2 hours, phase contrast |
| (b) 1 day, phase contrast | (f) 4 days, phase contrast |
| (c) 6 days, phase contrast | (g) 12 days, phase contrast |
| (d) 6 days, polarized light | (h) 12 days, polarized light |

smaller and more uniform. This difference is more apparent with thin slide preparations.

It is obvious that there are far too many crystals in the field to give good definition as to form. Hence slides were prepared by the other techniques, which show greater detail.

*Observations with Technique (B).* Photomicrographs of lard and rearranged lard each taken of the same field for various periods of storage at 24.5°C. are shown in Figure 2. The progressive development of the crystals of lard is clearly shown (a, b, c) with phase contrast. In (c) after six days the long delicate thread-like needles are clearly shown, but in polarized light (d) they are hardly noticeable. Some of the crystal structures do not appear to have changed much after the first day. The long filaments probably represent glycerides whose melting points in the medium are only slightly above the temperature of storage and therefore come out of solution much more slowly.

By contrast, the crystals from rearranged lard (e, f, g, h) showed only slight change even after 12 days storage under the same conditions. The crystal aggregates are more uniform and much smaller than those of lard after six days.

*Observations with Technique (C).* A slide prepared with a diluted sample (Figure 3) shows even more clearly the development of several different crystal formations in lard. The clusters of long thread-like crystals appear last and tend to obscure some of the star-shaped forms. The pictures were taken of the same field during storage.

For comparison, a slide was prepared in a similar manner with rearranged lard, and the same field was photographed at different time-intervals. Again, as in the preceding examples, there was little change in appearance of the crystals during storage. However the clusters of needles are larger and better developed than those by the other methods of slide preparation.

*Miscellaneous Observations.* In Figure 4 (a, b, c) are shown miscellaneous pictures of crystal formations found in different areas of the slides prepared with lard. In Figure 4 (d) the particular field of this slide prepared from rearranged lard appears to have all crystals of the same type. Also shown in Figure 4 (e, f) are photomicrographs of a low melting disaturated glyceride fraction isolated from lard. The crystals of this particular fraction exhibit a well-defined extinction cross in polarized light (f).

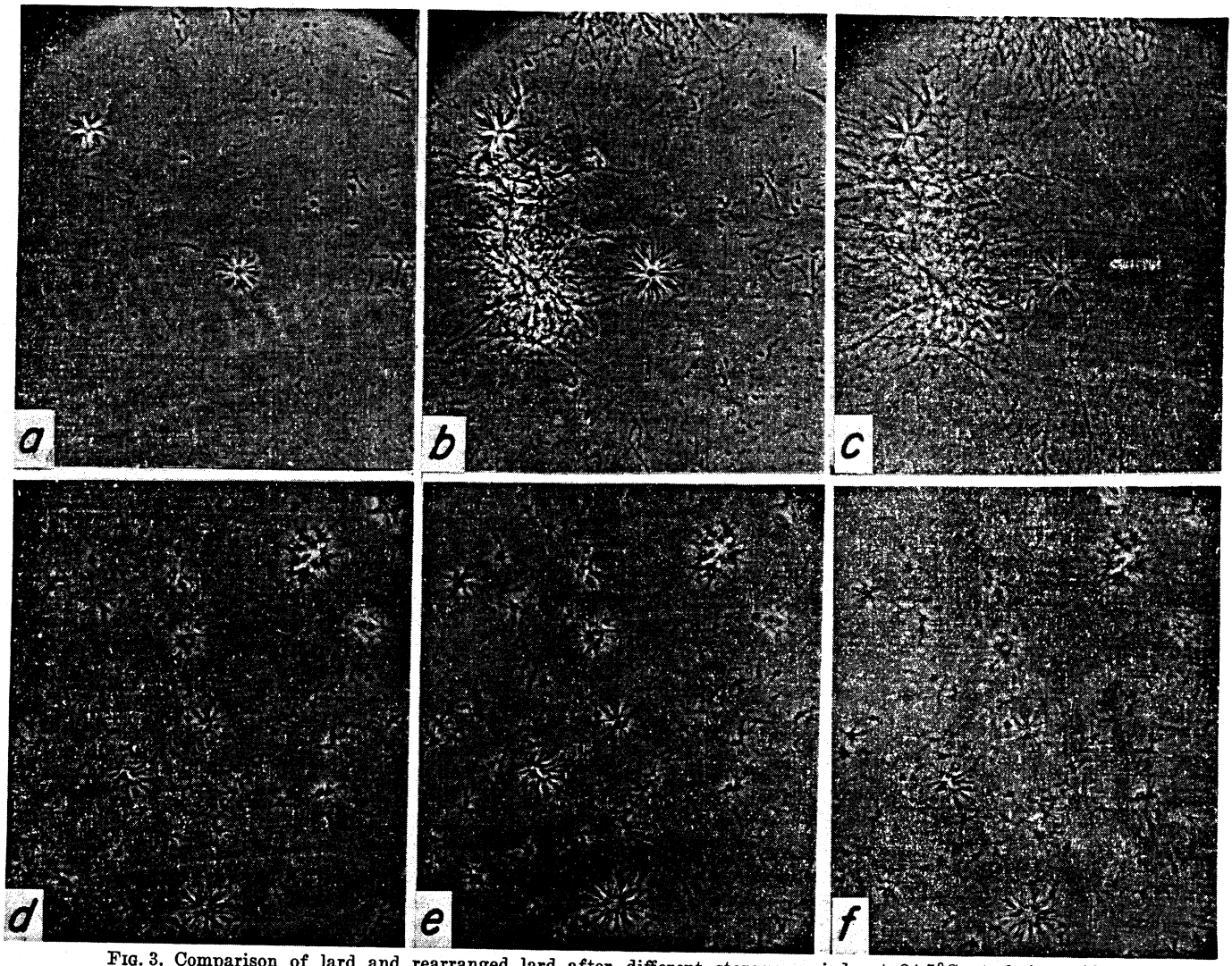


FIG. 3. Comparison of lard and rearranged lard after different storage periods at 24.5°C., technique (C).
Lard, phase contrast, 100 x magnification
(a) 7 hours, (b) 2 days, (c) 5 days
Rearranged lard, phase contrast, 100 x magnification
(d) 7 hours, (e) 2 days, (f) 5 days

Further work is under way to examine more extensively a number of purified types of glycerides, especially with the view of determining whether they can be related or identified with any particular crystal habit or physical property of a fat.

## Summary

Phase contrast and polarized light microscopy were employed to observe the crystalline nature of lard and rearranged lard. Special attention was given to the technique of specimen preparation, controlled conditions of tempering the sample, and photographic reproduction of crystal appearance. Photomicrographs are reproduced to show the difference in appearance of the crystals from lard and rearranged lard when both polarized light and phase contrast are employed. The peculiar long, thread-like crystals characteristic of lard are more clearly shown with phase contrast.

## REFERENCES

1. Anikin, A. G., and Ravich, G. B., Doklady Akad. Nauk. S.S.S.R., *68*, 309 (1949).
2. Bailey, A. E., "Melting and Solidification of Fats," pp. 18, 20, 21, Interscience Publishers Inc., New York, 1950.
3. Bennett, A. H., Osterberg, H., Jupnik, H., and Richards, O. W., "Phase Microscopy," John Wiley and Sons, New York, 1951.
4. Clarkson, C. E., and Malkin, T., J. Chem. Soc., London, 666 (1934).
5. Carter, M. G. R., and Malkin, T., J. Chem. Soc., London, 577 (1939).
6. Carter, M. G. R., and Malkin, T., J. Chem. Soc., London, 1518 (1939).
7. Efremov, N. N., Ravich, G. B., and Vol'nova, V. A., Izvest. Sektora Fiz-Khim, Anal. Inst. Obshchei i Neorg. Khim., Akad. Nauk., S.S.S.R., *16*, No. 3, 142 (1948).
8. Greene, L. W., Oil and Soap, *11*, 31 (1934).
9. Greene, L. W., Chemist-Analyst, *25*, 79 (1936).
10. Greene, L. W., Amer. J. Pharm., *109*, 67 (1937).
11. Greene, L. W., Amer. J. Pharm., *110*, 492 (1938).
12. Greene, L. W., Cotton and Cotton Oil Press, *39*, No. 14, 3 (1938).
13. Greene, L. W., Drug and Cosmetic Industry, *43*, 156 (1938).
14. Greene, L. W., Perfumery and Essential Oil Record, London, *30*, 309 (1939).
15. Greene, L. W., Amer. J. Pharm., *119*, 59 (1947).
16. Greene, L. W., Amer. J. Pharm., *121*, 91 (1949).
17. Hellman, N. N., Zobel, H. F., and Senti, F. R., J. Am. Oil Chemists' Soc., *32*, 110 (1955).
18. Hessler, W., Fette u. Seifen, *55*, 529 (1953).
19. Hessler, W., Fette u. Seifen, *55*, 596 (1953).
20. Hoerr, C. W., and Waugh, D. F., J. Am. Oil Chemists' Soc., *32*, 37 (1955).
21. Malkin, T., Shurbagy, M. R., and Meara, M. L., J. Chem. Soc., London, 1409 (1937).
22. Malkin, T., and Meara, M. L., J. Chem. Soc., London, 103 (1939).
23. Malkin, T., and Meara, M. L., J. Chem. Soc., London, 1141 (1939).
24. Mehlenbacher, V. C., Oil and Soap, *13*, 277 (1936).
25. Quimby, O. T., J. Am. Chem. Soc., *72*, 5064 (1950).
26. Ravich, G. B., Tsurinov, G. G., Vol'nova, V. A., and Petrov, N. P., Bull. Acad. Sci. U.S.S.R., Classe sci. chim., *6*, 581 (1945).
27. Ravich, G. B., and Vol'nova, V. A., Doklady Akad. Nauk. S.S.S.R., *66*, 417 (1949).
28. Reinders, W., Doppler, Ch. L., and Oberg, E. L., Rec. Trav. Chim., *51*, 917 (1932).
29. Reynolds, P. M., and Verma, A. R., Nature, *171*, 486 (1953).
30. Rosevear, F. B., J. Am. Oil Chemists' Soc., *31*, 628 (1954).
31. Weitkamp, A. W., J. Am. Chem. Soc., *67*, 447 (1945).

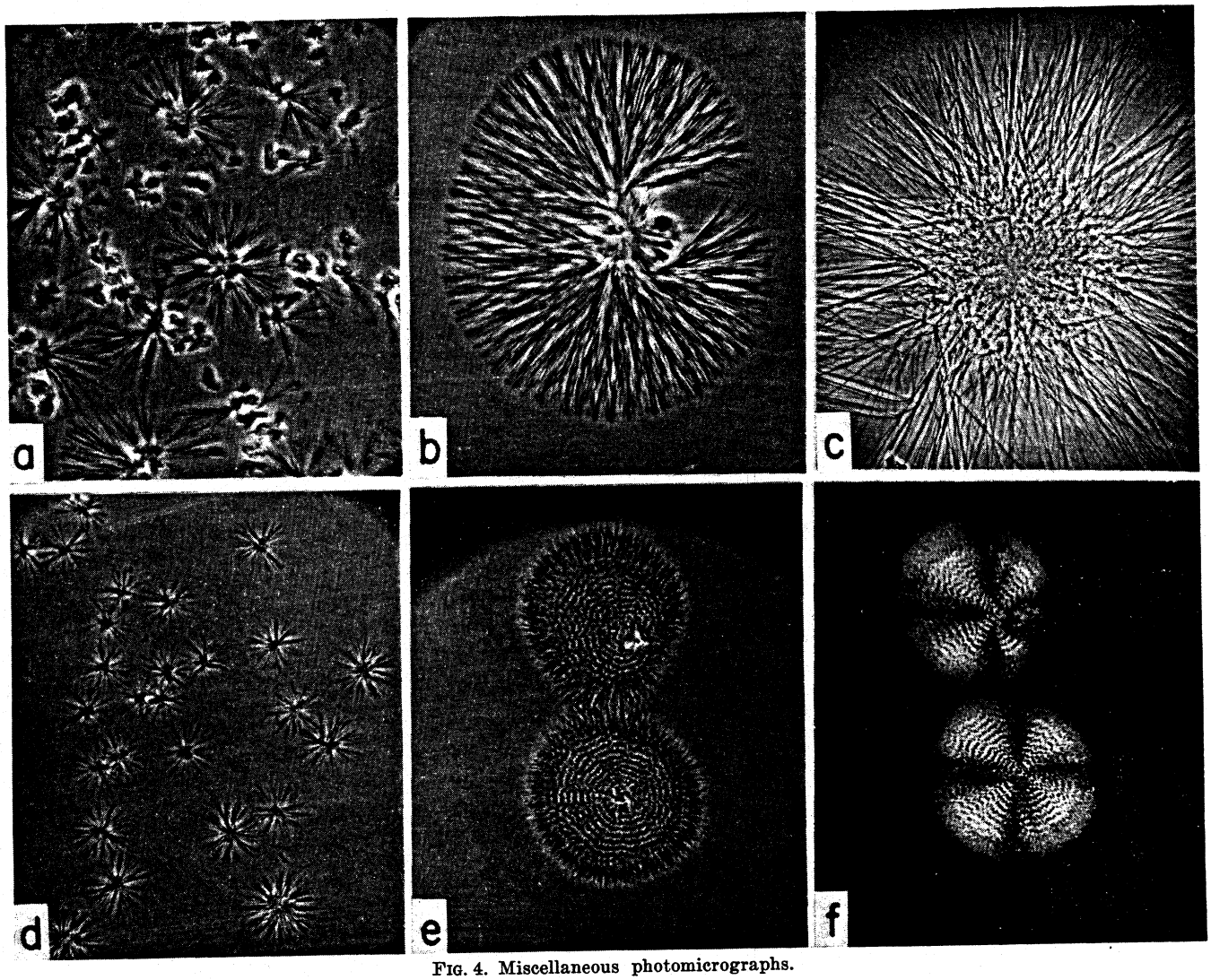


FIG. 4. Miscellaneous photomicrographs.

(a, b, and c) from lard, technique (B), 400 x magnification, phase contrast.
(d) from rearranged lard, technique (C), 100 x magnification, phase contrast.
(e) low melting di-saturated glyceride fraction from lard, technique (C), 100 x magnification, phase contrast.
(f) low melting di-saturated glyceride fraction from lard, technique (C), 100 x magnification, polarized light.